



Přírodovědecká
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Jihočeská univerzita
v Českých Budějovicích
University of South Bohemia
in České Budějovice

OPPONENT'S REVIEW ON BACHELOR/DIPLOMA* THESIS

Name of the student: Magdalena V Baranyi

Thesis title: Hafnium Chloride, an Alternative Staining Reagent for Biological Electron Microscopy

Supervisor: RNDr. Marie Vancová, Ph.D.

Referee: RNDr. Jiří Týč, Ph.D.

Referee's affiliation: BC CAS, Parasitology institute, České Budějovice

	Point scale ¹	Points
(1) FORMAL REQUIREMENTS		
Extent of the thesis (for bachelor theses min. 18 pages, for masters theses min. 25 pages), balanced length of the thesis parts (recommended length of the theoretical part is max. 1/3 of the total length), logical structure of the thesis	0-3	3
Quality of the theoretical part (review) (number and relevancy of the references, recency of the references)	0-3	3
Accuracy in citing of the references (presence of uncited sources, uniform style of the references, use of correct journal titles and abbreviations)	0-3	3
Graphic layout of the text and of the figures/tables	0-3	3
Quality of the annotation	0-3	2
Language and stylistics, complying with the valid terminology	0-3	1
Accuracy and completeness of figures/tables legends (clarity without reading the rest of the text, explanation of the symbols and labeling, indication of the units)	0-3	2
Formal requirements – points in total		17
(2) PRACTICAL REQUIREMENTS		
Clarity and fulfillment of the aims	0-3	2
Ability to understand the results, their interpretation, and clarity of the results, discussion, and conclusions	0-3	2
Discussion quality – interpretation of the results and their discussion with the literature (absence of discussion with the literature is not acceptable)	0-3	1
Logic in the course of the experimental work	0-3	2
Completeness of the description of the used techniques	0-3	3

* Choose one

¹ Mark as: 0-unsatisfactory, 1-satisfactory, 2-average, 3-excellent.

Experimental difficulty of the thesis, independence in experimental work	0-3	3
Quality of experimental data presentation	0-3	2
The use of up-to-date techniques	0-3	3
Contribution of the thesis to the knowledge in the field and possibility to publish the results (after eventual supplementary experiments)	0-3	2
Practical requirements – points in total		20

POINTS IN TOTAL (MAX/AWARDED)	48	37
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Comments of the reviewer on the student and the thesis:

The thesis focusses on the important topical issue of using alternative stains to Uranyl acetate that is slowly being banned due to its radioactivity issues. The thesis has a standard structure and appropriate length. The citations of previous work are used well with few exceptions. What this thesis suffers from the most is the stylistic style. The style does not comply with scientific text standards but rather the language teachers' standards at primary and secondary schools. The text is complicated and really hard to read. Long sentences and use of fancy words go against the clarity of the message. The scientific text must be easy to read and follow, and the main point is to deliver a clear message. Rule of thumb says that if the word can be omitted without the change of meaning of the sentence, it should be omitted. I consider bachelor theses as the first try of this form so it would be good to learn from these mistakes and criticism and use it hopefully for writing the master thesis in the future.

The chemical part of the thesis is definitely more robust than the biological and electron microscopy part, which I believe results from student focus on biochemistry. I consider this as OK. However, some basic stuff should be clarified to show that the student understands what she was doing during her thesis.

Suggestions and questions, to which the student has to answer during the defense. Mistakes, which the students should avoid in the future:

QUESTIONS:

- Is there any particular reason why did you use different temperature curves for FS1 and FS2 protocols? Do you think that having them both the same would be beneficial for your work?

- As you want to compare the UA and HfCl₄ and you used the UA for counter staining in both cases. Do you think that using HfCl₄ also for counter staining will make any difference?

Some comments:

Major issues:

- **1.2 page 2** "In TEM, the electron beam can be emitted thermionically into vacuum from a small filament tungsten or microscopic LaB₆ crystal, which is connected to a high voltage source (Kobayashi et al., 1992; Rukari & Babita, 2013). Also, field emission is a possible mode, where a strong electrostatic field is used to induce electron emission. "

I am afraid that this does not make any sense and I am not sure if the student understands the principle of electron sources in the EM. I would appreciate it if the student could present different electron sources used in the EM and describe the principle of electron extraction from them. As this is really the absolute basic.

- **1.3 page 3** "Volume Electron Microscopy was initially developed and used to image parts of the central nervous system in order to better the reconstruction when it comes to 3D data of tissue obtained. It

was found that due to the small dimensions of synaptic vesicles, great resolution in the z-axis was obtained (Denk & Horstmann, 2004). "

How can volume EM improve the reconstruction of 3D data? It provides 3D data in the first place. How can synaptic vesicles improve the z-axis resolution?

1.4 page 3 "Biological materials, analyzed with TEM, need to be manipulated to preserve the cell's structure in native conditions (Fitting Kourkoutis, Plitzko & Baumeister, 2012)."

How can be the cells preserved in native conditions inside the TEM?

- **1.5 page 7** "Cryofixation is usually performed for objects with depths up to 200 nm via the process of High Pressure Freezing (HPF) (Moor & Riehle, 1968)."

Could you verify the size of the sample that can be frozen by HPF?

- **3.2.3.1 and 3.2.3.2 page 20 and 21 respectively.** Both protocols contain both UA and HfCl₄. This is inconsistent with the other sections of the thesis. Could the student verify the protocols?

- **3.2.6 page 24** "For later being able to better distinguish the staining efficiencies of UA and HfCl₄ in the OTOSTaining process, a number of grids were left not counterstained, others were only counterstained with either UA or LC or with both solutions."

This (4 counterstaining conditions) sounds to be a good control. However, in the result section, you are using only those that were stained with both UA and LC or in one case only with UA. So you should either omit the rest from here or include the results in the result section.

- **Figure 13. page 26** "Sections through *I. ricinus* acinar duct. specimen was en-bloc stained with uranyl acetate according to protocol 1. Images were counterstained with UA and LC respectively and imaged in TEM JEOL 1010."

Respectively is being used if there are 2 different images obtained under 2 different conditions. In this case, one would be counterstained only with UA and one only with LC. Is this the case? If so, which one is which one? There are 3 images in Fig.13.

- **4.2.1 page 27-28.** Fig. 14.C and 14.D are not mentioned in the text. Fig. 14.C is only referred to twice during the discussion on page 31

- **5. Discussion** Whole paragraphs are not discussion but rather should be part of the introduction. For example, the paragraph about UA and radioactivity is actually something that I was missing in the introduction where it was only briefly mentioned. Also, the final paragraph about Cyclometalated iridium is not in the form of discussion but rather a sum of information about the chemical. For discussion, one should compare Cyclometalated iridium to the Hafnium chloride, how they differ what each should be used for etc.

Minor issues:

- English. The student is not a native speaker, and in general, her written English is above the average. Some mistakes are still present, and there are some inconsistencies. For example "the TEM" is being used without or with the article randomly.

- **1.1. page 1** "Electron microscopy is a microscopy technique said to have been first established in Germany by a team of physicists in the 1930ies (von Ardenne, 1938; Mulvey, 1967)."

If there is a citation than this is valid and truthful information. The phrase "Said to" should not occur in the scientific text.

- **1.1. page 1** "It functions due to electrons having a much shorter wavelength than visible light, as well as the resolution increasing if the electrons are at higher speed, which is feasible by increasing the acceleration voltage (Flegler, Heckman, Klomparens, 1993)."

Although I believe I know what student wanted to say this sentence does not make any sense. (this is one example out of quite a few)

- **1.1.-1.2 page 1 and 2** Although transmission electron microscopy has its own chapter 1.2, the Scanning electron microscopy has only paragraph in the introduction.

- **1.2 page 2** "Protein ultrastructures at close to atomic level resolution have been made visible in the past years (Cortese, Diaspro & Tacchetti, 2009)."

This citation refers to a review about Advanced Correlative Light/Electron Microscopy: Current Methods and New Developments Using Tokuyasu Cryosections. However, here the citation of single-particle analysis would be appropriate.

- **1.4 page 6** "In order to infiltrate the samples before freezing, the samples are placed in a glucose or sucrose phosphate buffer solution in an ice bath for up to 30 min, wherein the molar concentration of the sucrose is 2,3 M. Fixed and infiltrated samples are then to be frozen on liquid nitrogen (Tokuyasu, 1973; Griffiths et al., 1984)."

In order to infiltrate the sample with what and why? How are you freezing the samples **on** the liquid nitrogen?

- **1.6.1 page 9** "Metallic osmium appears black on the tissue and thereby is used as a secondary stain. In volume EM, strong contrast for biomembranes is needed."

Are you sure, that Osmium stain always appears black in the EM? Especially considering the volume SEM that you mention in the following sentence.

- **1.9.1 page 13** "The binding affinity of these complexes is highly dependent on the surrounding pH, as well as the UA concentration. In TEM, the concentration is often times high enough for the complexes binding to positive groups, to be more likely."

Could you please rephrase the second sentence, so it makes sense?

- **3.2.3.2 page 20** "This is followed by a temperature rising period of 12h from -90°C to 10°C, with the warming rate being approximately 8.3°C/h."

Based on the table and graph provided I believe that the temperature here should end at -30°C, not 10°C

- **3.2.4 page 22** "The pure resin infiltration was not performed on a shaker, but in a desiccator, to remove any air bubbles that might have been present, since high humidity may disturb the polymerization."

How can the desiccator be used to remove air bubbles from your sample?

Conclusion:

In conclusion, I recommend the thesis for the defense and I suggest the grade very good

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In **České Budějovice** date 20.1.2020



² You can suggest a grade, which can be modified during the defense based on the presentation. However, if the reviewer is not present at the defense, the grade will not be counted. Grades: excellent (1). Very good (2), Good (3), Unsatisfactory/failed (4).